Infection Studies with Watermelon Wilt Caused by *Fusarium niveum* EFS.

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AGRICULTURAL EXPERIMENT STATION
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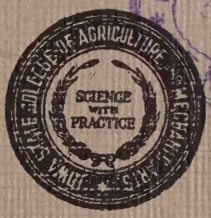
BOTANY AND PLANT PATHOLOGY SECTION



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SUMMARY

Watermelon wilt, caused by *Fusarium niveum,* is a serious disease in Iowa and in many other sections of the United States. The present Iowa acreage is less than 10 percent of the acreage before wilt became a factor.

Three heretofore undescribed seedling symptoms are described. These are: seedling rot, damping off and stunting.

The organism may cause lesions on any of the roots. These are at first water-logged and become discolored in advanced stages. The lesions vary in length from a trace to 28 centimeters.

Infection may be induced thru the use of infested soil, by means of spore suspensions of the organism injected with a hypodermic needle into the hypocotyl and by the insertion of the mycelium into wounds.

It is probable that the organism enters the host thru root hairs, root injuries and thru the epidermis of the hypocotyl.

Fusarium niveum has been recovered from primary, secondary and tertiary roots, from stems, petioles, leaves, peduncles, melon flesh and seeds of infected plants.

The organism has also been recovered from and observed in the vascular tissues of what appeared to be normal, healthy plants.

The organism flourishes at high temperatures. Its optimum on potato dextrose falls between 24 and 32°C., the minimum below 12°C. and the maximum above 35°C.

The organism flourishes on acid media, the optimum falls between pH 4.6 and 6.0 and the minimum below 3.0.

With proper technique, seedling susceptibility in the greenhouse gives a good index of the susceptibility to be expected in the field.

Infection Studies with Watermelon Wilt Caused by *Fusarium niveum* EFS.

By D. R. Porter*

Watermelon wilt has been known since 1894, but the problem of infection is still unsolved, and the interdependence of infection, environmental conditions and host relationships is not well understood. Smith (18) in 1894 showed that watermelon wilt was caused by *Fusarium niveum* EFS., a vascular parasite which entered the plant from infested soil. The following year he (19) found macroconidia and chlamydospores, to which he referred as external conidia. In 1899 he (21) gave an excellent report of the morphology, vitality, longevity, pathogenicity and dissemination of the fungus, a description of some of the seedling and field symptoms and an account of the relation of the fungus to the host.

Since the publication of Smith's investigations Orton (11), (12), (13), (14), (15) and Taubenhaus (23) have contributed to our knowledge of watermelon wilt. Orton's work relates chiefly to the development of resistant strains. He developed the Conqueror, a wilt resistant strain resulting from a cross between the stock citron and the susceptible commercial variety Eden. Unfortunately this strain has not proven sufficiently fixed as to type and resistance to find a place in practice. Taubenhaus (23) working in Texas was able to show that the organism is widely distributed at different depths in the soil, that it spreads more rapidly in soil where manure is used than where commercial fertilizer is applied, and that there is evidence that the pathogen is more active during warm than cool seasons.

In connection with studies started four years ago at this station on the development of resistant strains, it was soon learned that further work was necessary on infection phenomena before resistance or susceptibility could be accurately measured.

Intensive investigations have been carried on since March, 1926, in the field and laboratory at Conesville, Muscatine County, Iowa, during the summer and in the plant pathological laboratories of the Iowa Station at Ames during the winter.

The purpose of this preliminary paper is: (1) to record studies relating to conditions surrounding infection, (2) to describe more fully the symptoms produced, (3) to point out some methods of infection and (4) to indicate the response of the host to the attack of the parasite.

*The writer wishes to express his indebtedness to Drs. I. E. Melhus and J. C. Gilman for critical reading of the manuscript, and to Dr. Melhus for the definition of the problem and for helpful criticism and suggestions given thruout the course of these investigations.

HISTORY OF THE DISEASE IN IOWA

Smith (18) in 1894 reported the prevalence of watermelon wilt in the southern part of the United States. In 1897 he (20) reported that the wilt disease was prevalent from South Carolina to Texas and was the most serious disease with which watermelon growers had to contend.

Watermelon wilt is apparently a disease of long standing in Iowa. It is quite generally believed by the older growers, some of whom have been growing watermelons in Iowa for over 50 years, that the disease was present in Iowa before 1900.

Altho there is no way of ascertaining absolutely the exact date that this disease was introduced into Iowa, the carlot shipments have continually fallen off since about 1915.

Since this disease became serious in the Muscatine and Conesville sections the annual production has never been more than half of what it was before the introduction of watermelon wilt. The acreage in 1900 was about 8,000. In 1926 it was 641.

Carlot shipments of watermelons from the three most important watermelon growing counties in 1924 and 1926 were 14 and 5 percent, respectively, of those from the same territory in 1921. This decline may be charged, mostly, to wilt.

In addition to the decline in acreage and carlot shipments, another form of loss has resulted. While the watermelon acreage has fallen rapidly since the introduction of wilt, much of the acreage on Muscatine Island once devoted to watermelons is no longer under cultivation. The same is true of a large acreage near Nichols and Conesville. Much of this land is adapted only to the production of such crops as watermelons, sweet potatoes and cantaloupes, but the abandoned acreage is much too large to be devoted either to sweet potatoes or cantaloupes under present conditions and, as a result, many fields lie fallow. Fields that once produced good crops of melons have been idle for 16 years. The land values have dropped materially and the watermelon section has lost its air of prosperity, a condition that may be directly charged to watermelon wilt.

SYMPTOMS ON SEEDLINGS

One symptom of seedling infection was first described by Smith (20), who, by infesting soil with a pure culture of *Fusarium niveum*, was able to induce wilting of over 500 seedlings. He pictures the most common seedling symptom, characterized by drooping of the cotyledons. This symptom was also noted by Taubenhaus (23) and Orton (15). Additional symptoms in the order of their appearance are seedling rot, damping off and stunting, which commonly appear under greenhouse conditions, altho not so often as the drooping of the cotyledons.

SEEDLING ROT

Seedling rot is typically a rot of the hypocotyl, which kills the seedling shortly after germination and before it emerges from the soil. This rot was first observed in a series of experiments in which seed had been planted in artificially infested soil. Altho seed from the same lot planted in steam sterilized soil produced a perfect stand, that planted in the artificially infested soil seldom produced over a 20 percent stand and, in some of the pots, the stand was nil. In such cases examination of the seeds showed that practically every one had germinated in the infested soil, but that the root and hypocotyl had become discolored. This discoloration in the early stages was purely external, the epidermis being rotted or the secondary roots being destroyed. As the fungus progressed the cortex became invaded and exhibited slight browning, the epidermis becoming more intensely discolored. By the time the fungus reached the vascular tissues the epidermal and cortical tissues were blackened and in many cases softened. In the event that the seed germinated sufficiently to free the cotyledons while still under ground, these leaves also became invaded by the fungus. Infection in such cases probably took place thru the epidermis, causing necrosis of the external tissues. The fungus probably entered the vascular system and killed the seedling before emergence. In order to determine this point further a box 20 by 14 by 10 inches was divided into three equal compartments. One compartment was filled with steam sterilized soil, one with lightly infested soil and one with heavily infested soil. Fifteen seeds previously disinfected for 15 minutes in a 1-1000 solution of mercuric chloride were planted in each of the three compartments on January 22, 1927. By January 29 there was a good stand in the steam sterilized soil and in the lightly infested soil, while only one plant had emerged in the heavily infested soil. This plant lived for nearly a month before it suddenly wilted. The plants in the lightly infested soil wilted very slowly at the rate of about one plant every four or five days.

Fig. 1 illustrates the effect of seedling rot caused by *Fusarium niveum*. Compartment "A" contained steam sterilized soil, and the plants in this compartment remained healthy thruout the experiment. Compartment "B" contained soil heavily infested with *F. niveum* and only one plant emerged. Examination of the balance of the seeds planted in this compartment, 14 in number, showed that 12 had germinated, but the seedlings had been killed by the fungus before they had emerged. The symptom on these 12 seedlings was characteristic necrosis of the epidermal and cortical tissues. Microscopic examination revealed the presence of mycelium in the cortical and vascular tissues and *F. niveum* was recovered in every case.

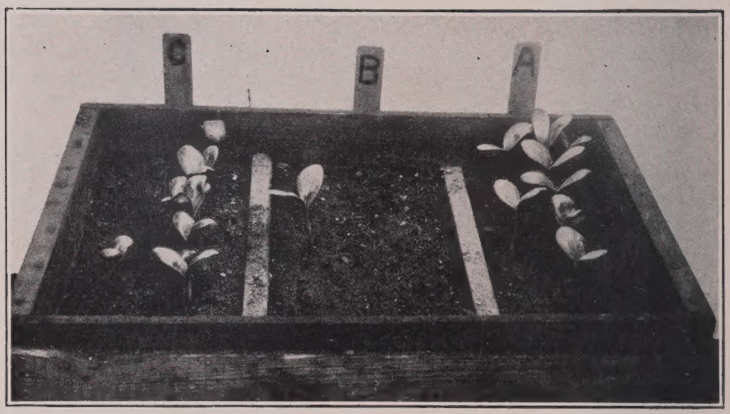

Fig. 1. Seedling rot, caused by *Fusarium niveum*. Compartment A—steam sterilized soil, B—heavily infested soil and C—lightly infested soil. 12 of 15 seeds planted in compartment B germinated, but the seedlings were killed by the fungus before emergence.

STUNTING

Stunting was first observed in the field during the summer of 1926. Many plants growing in naturally infested soil exhibited stunting or dwarfing. Such plants appeared unthrifty as tho plant food were lacking. Such vines showed no sign of wilting, but in many cases *Fusarium niveum* was recovered from them. This led to the suspicion that *F. niveum* was capable of inducing stunting. A striking case of stunting appeared in the seedling rot experiment referred to above (fig. 1) in which plants were growing in steam sterilized soil in compartment "A" and in

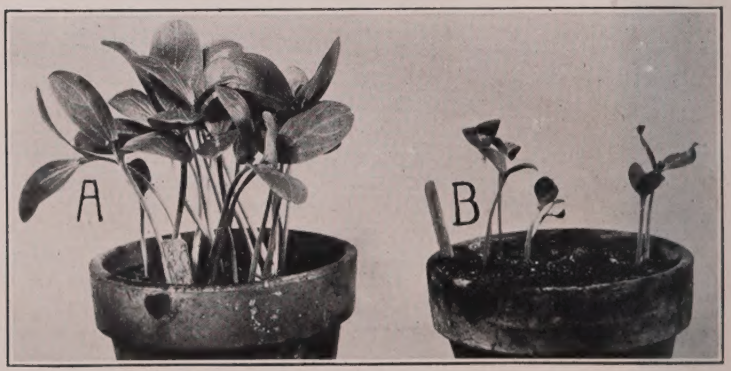

Fig. 2. Stunting of watermelon seedlings caused by *Fusarium niveum*. A—steam sterilized soil perfect stand and no wilting. B—artificially infested soil, 34 percent stand, and those plants which emerged were stunted.

lightly infested soil in compartment "C." Stunting was evident in compartment "C" as soon as the first true leaves formed. The true leaves formed earlier in the steam sterilized than in the infested soil, the hypocotyl elongated more rapidly and the cotyledons attained greater size. Stunting became more pronounced as the plants grew older, being manifested by shortened internodes and smaller leaves until after 28 days when the average length of the vines in steam sterilized soil was 21 centi-

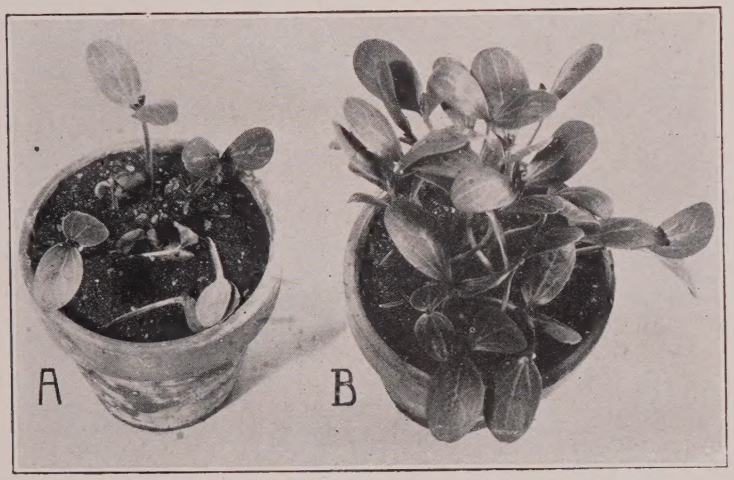

Fig. 3. Damping off, caused by *Fusarium niveum*. A—infected soil, 60 percent stand; 40 percent killed by damping off. B—steam sterilized soil, perfect stand, no wilting, no stunting and no damping off.

meters that of the vines in infested soil was only 16 centimeters. Orton (11) has shown *Fusarium vasinfectum* capable of causing stunting of cotton plants; Cromwell (3) has shown that *F. tracheiphilum* causes stunting of soybean plants; and Barker (1) has shown that *F. lini* may induce stunting of flax plants. Stunting is shown in fig. 2.

DAMPING-OFF

The third undescribed symptom of watermelon wilt is damping off. It was first observed in an experiment in which watermelon seedlings were growing in soil which had been taken from areas in the field at Conesville where all the plants had wilted. Where damping off occurred the hypocotyl first became water soaked at the ground line, following which the epidermal and cortical tissues became invaded and weakened sufficiently to cause the plant to fall over. When this symptom first appeared it was thought not to be due to *Fusarium niveum*, but this was the only organism recovered from seedlings so infected.

In order to determine further whether or not *Fusarium niveum* would induce damping off of watermelon seedlings, steam sterilized soil was infested with a culture of the organism growing on sterile oats. The inoculum was used at the rate of 10 grams to each 5-inch pot of soil. Seeds of the variety Halbert Honey were disinfected for 15 minutes in a 1-1000 solution of mercuric chloride, washed twice in sterile distilled water and planted in 10 pots at the rate of 15 seeds per pot. Controls consisted of similar seeds planted in steam sterilized soil. Fig. 3 represents what had happened after 18 days. The steam sterilized soil produced practically a perfect stand, while the infested soil produced only nine plants, six of which showed the damping off symptom, while three were stunted. This experiment was repeated once more with practically identical results.

It has not been definitely determined where infection takes place in order to induce damping off. Possibly it takes place thru the roots, causing necrosis of the tissues at the ground line. The other possibility is that infection takes place thru the epidermis at the soil surface, causing necrosis of the epidermal and cortical tissues and causing the plant to fall over. Rosen (17) has shown that *Fusarium vasinfectum* may cause damping off of cotton seedlings, while Barker (1) found that *F. lini* would induce damping off symptoms on flax plants.

WILTING

The fourth symptom on seedlings is the one first described by Smith (21). He states: "The gross symptoms are those of a plant transpiring freely and insufficiently supplied with water,

Fig. 4. Seedling wilt, caused by *Fusarium niveum*. A—steam sterilized soil, no wilting, damping off, stunting or seedling rot. B—infested soil, 70 percent stand; every plant showing symptoms of seedling wilt.

altho at the same time there is an abundance of moisture in the soil." This symptom is first manifested by drooping and flaccidity of the cotyledons. Simultaneously with the appearance of these two symptoms the cotyledons gradually lose their dark green color and become pale green with a faint tinge of yellow. Soon these leaves droop and wither. Often, however, the hypocotyl may remain turgid for several days after wilting of the cotyledons. This symptom is shown in fig. 4. During early June, 1927, this wilting symptom was observed in the field at Conesville and Muscatine. Counts on 600 plants on June 3 showed less than 1 percent wilt, while on June 14, counts on 1100 plants showed 4 percent wilt. A decided rise in temperature on June 4 and 5 suggests the reason for the sudden increase in the rate of wilting.

SYMPTOMS ON OLDER PLANTS

The symptoms produced on above ground parts of plants past the seedling stage have been very well described by Smith (18), (21), Orton (15) and Taubenhaus (23). Little mention is made, however, of the symptoms which appear on the roots.

ROOT CANKERS

It often happens that apparently healthy root systems are found on vines which are entirely wilted. Upon closer examination, however, necrotic areas may almost always be found on some portion of the root system. Lesions may occur at any point on any of the roots down to a depth of 3 feet, being found quite often at a point where a root has sent out a branch. The necrotic area is first water logged, then turns yellow, brown or even black in later stages. The discoloration may extend for only a short distance along a root or it may extend a foot or more. Often several secondary or tertiary roots show visible infection, but more often only one very short rootlet is found infected even tho the vines are entirely wilted. The writer (16) called attention to the symptoms on the roots in an earlier publication.

FOLIAGE WILTING

The vine may wilt suddenly and die, or it may begin to wilt during the heat of the day and recover over night. Alternate wilting and recovery may be repeated for several days before the plant dies.

METHODS OF INFECTION

The methods by which *Fusarium niveum* gains entrance to the host are not well understood. Smith (21) merely states that the fungus enters the plant thru the underground parts, while Orton (15) states that it enters thru the small roots. It is not definitely

known how infection takes place, altho circumstantial evidence indicates that the fungus enters chiefly thru the roots.

SOIL INFESTATION

Infection of watermelon seedlings by *Fusarium niveum* may be obtained in many ways, the most common of which is thru the use of infested soil. Soil may be artificially infested by mixing soil and culture of *F. niveum*, or by applying the culture either below or at the soil surface. The inoculum may be supplied in the form of spore suspensions, agar cultures or giant cultures of the organism growing on sterilized oats. The results of artificial soil infestation are shown in figs. 1, 2, 3 and 4.

HYPODERMIC INOCULATIONS

Hypodermic inoculations with a heavy spore suspension of *Fusarium niveum* are effective in inducing infection. Typical wilt symptoms follow such inoculations. Seedlings grown for two weeks in sterile soil were injected with 3 cc. of a heavy spore suspension of *F. niveum* on December 3, 1926. Inoculations were made in the hypocotyl above and below the soil surface and about two centimeters back of the growing tip. Controls consisted of like inoculations with sterile distilled water. After 30 days, 66 percent of the hypodermic inoculations in the hypocotyl had caused wilting, while 36 percent of the growing tip inoculations had produced positive results. All the controls remained healthy.

THRU WOUNDS

Infection occurs very slowly and very seldom when the above ground parts of seedlings or of plants past the seedling stage are injured and mycelium and spores of the organism inserted in the wound. Wounding of underground parts either of seedlings or of older plants in the field often facilitates infection.

HYPOCOTYL INFECTION

Infection may occur naturally thru the hypocotyl. This was demonstrated by many experiments like the following: watermelon seeds were disinfected for 10 minutes in a 1-1000 solution of mercuric chloride; then washed three times and soaked over night in sterile distilled water; and planted in sterile soil in 5-inch greenhouse pots. These pots were watered with sterile distilled water and the plants allowed to grow until they were approximately 5-inches high. At the end of this time the top inch of soil was removed from each pot and glass tubes with an inside diameter of one-quarter inch were inserted into the soil. This precaution made it possible to irrigate the root systems by pouring water down the glass tube. A mixture of 8 parts of parowax to 1 part of beeswax, melted together and cooled to a temperature of 52 C., was poured over the soil until a layer about ½-inch thick was formed. A layer of artificially infested

soil was then placed on top of the hardened wax. The infested soil also was watered with sterile distilled water. Controls were made as above outlined except that steam sterilized soil was substituted for infested soil.

From a total of 63 plants growing in 15 pots and comprising three identical sets of experiments, 48 had wilted in 30 days after soil infestation. Infection, then, probably does not always take place thru the roots.

ROOT HAIRS

Infection probably takes place thru the root hairs before secondary roots form. This was evident from an experiment in which watermelon seeds, previously disinfected in a 1-1000 solution of mercuric chloride for 10 minutes, were sprouted under sterile conditions. Following this, with the appearance of the first root hairs, the seedlings were immersed in a spore suspension of *Fusarium niveum* for a few minutes and then removed to sterile moist chambers. They were then incubated at 30°C. for 48 hours. Following this they were disinfected for periods ranging from 1 to 5 minutes in a 1-1000 solution of mercuric chloride and planted in steam sterilized soil in greenhouse pots.

The working hypothesis in this experiment was that root hair infection and subsequent vascular penetration probably took place rather promptly after exposure of the root hairs to infection. This being the case, disinfection of such exposed seedlings in mercuric chloride would kill any spores still remaining on the exterior. Then, if plants so treated would later wilt when grown in steam sterilized soil, it would be evident that penetration of the root hairs and subsequent tissue invasion by the organism had probably occurred. The uncertainty connected with an experiment of this kind lies in the possible ineffectiveness of mercuric chloride which might not kill all the spores on the exterior of the exposed seedling. If such were the case, root hair infection would not be proved by this type of experiment.

One hundred percent of the plants produced by seedlings exposed to a spore suspension, in the manner just described, wilted after 28 days. Where the seedings were disinfected for 1 minute following exposure to infection, 80 percent of the plants wilted after 28 days. Using a 2 minute disinfection, 100 percent wilt appeared, while 4 and 5 minute disinfections failed to prevent the appearance of seedling wilt; 75 percent of the plants produced by seedlings so treated wilted after 28 days.

While this evidence alone is not sufficiently complete to prove that root hair infection takes place, such evidence is supported by the fact that the mycelium of *Fusarium niveum* has been observed in root hairs of watermelon seedlings growing in infested soil and in the root hairs of seedlings exposed to a spore suspension of the organism. Since all the experiments with surface dis-

infected seedlings previously exposed to a spore suspension of the organism produced practically identical results, and since the fungus has been observed microscopically in root hairs of seedlings, root hair penetration is a likely method of infection.

PREVALENCE OF PATHOGEN IN DIFFERENT PARTS OF THE PLANT

During the course of these investigations hundreds of isolations were made from all parts of wilting and healthy appearing watermelon plants. The great majority of the fungus colonies proved to be Fusaria, but in order to make sure that *Fusarium niveum* was being constantly recovered it was deemed advisable to determine whether or not these cultures were pathogenic to watermelons. Accordingly representative cultures were selected from time to time, purified, tubed and brought to the laboratory at Iowa State College for pathogenicity trials. These trials were conducted by mixing one tube culture of the organism with the upper 2 inches of sterile soil in 5-inch greenhouse pots.

TABLE I. PATHOGENICITY OF CULTURES ISOLATED FROM ALL PARTS OF MELON PLANTS

Culture No.	Source	No. plants.	No. plants wilted	Percent wilt
1	Wilted runner of the preserving citron	9	8	77
3	Stem of wilting plant	8	8	100
4	Isolated by O. H. Elmer at Oskaloosa in 1925	10	10	100
10	Badly rotted secondary root	10	10	100
11	Healthy appearing Conqueror stem	8	8	100
12	Badly rotted secondary root	9	9	100
13a	Melon pedicels	5	4	80
13b	" "	7	7	100
13c	" "	9	9	100
13d	" "	7	7	100
13e	" "	9	9	100
13f	" "	6	6	100
13g	" "	4	4	100
13h	" "	7	7	100
13k	" "	7	7	100
13l	" "	9	9	100
13m	" "	4	4	100
13n	" "	7	4	57
13p	" "	7	4	57
13q	" "	3	3	0
13r	" "	6	6	43
14a	Melon flesh	10	10	100
14b	" "	7	7	100
14c	" "	7	7	100
14d	" "	2	2	100
14e	" "	8	5	40
14f	" "	3	3	100
14g	" "	6	6	100
14h	" "	4	4	100
15a	Melon seeds	6	6	100
15b	" "	8	8	100
15c	" "	8	8	100
15d	" "	7	7	100
15e	" "	6	6	100
15f	" "	7	2	28
16	Stem of dead plant	10	10	100
Check		86	0	0

Seeds of the Tom Watson variety were disinfected, washed, soaked over night and planted at the rate of 10 seeds per pot. Ten pots of steam sterilized soil were held as controls. Daily record was made of the emergence and rate of wilting in each pot. Table I shows the source of the various cultures used together with the pathogenicity of each after 45 days.

The data in table I show that, with one exception, all of the cultures tested proved pathogenic to watermelon seedlings. There was a marked difference in the time necessary for the various cultures to cause all of the plants to wilt. Culture No. 14f killed all the plants in 18 days, followed by Nos. 13h and 15a, both of which required 19 days. Most of the remaining cultures killed all the plants within 30 to 35 days, while a few required 45 days. Only one culture, No. 13p, failed to cause wilting.

Special attention should be called to the pathogenicity of culture No. 11. This was isolated from a runner of what appeared to be a perfectly normal, healthy Conqueror plant. Microscopic examination had revealed the presence of a fungus in the xylem vessels of many healthy appearing stems and roots, and many isolations from such were made. Fusaria appeared on the plates about half of the time and No. 11 was considered a typical culture from healthy plants. That a healthy appearing plant may harbor the fungus in its vascular system is proved by the pathogenicity of this culture. The plant from which culture No. 11 was isolated lived for nearly three weeks following the isolation until it was killed by anthracnose late in the season.

Cultures No. 15a to 15f, inclusive, were representative cultures obtained from the seeds of melons attached to wilting vines. Extreme care was exercised in making these isolations, and *Fusarium niveum* was obtained from seeds about 8 percent of the time. Over 1,300 seeds were plated out. Much of the literature indicates that watermelon wilt is seed borne, Fulton (7) having found such to be the case, and these results substantiate his findings. This fact will be discussed more fully later.

SPREAD IN HOST AND TISSUES INVADED

After some of the methods of host penetration by *Fusarium niveum* were determined, a study was undertaken of the tissues attacked and the rate of spread of the fungus after gaining entrance. *F. niveum* has been generally considered a vascular organism altho Smith (21) found that after plugging the water ducts the organism migrated into the parenchyma. Taubenhaus (23) states that the organism is confined to the vascular cylinder of all parts of the plant, the fruit apparently being an exception. The present investigations confirm those of Smith (21) who, in addition to finding the organism in the vascular system, found it in the parenchyma. During these investigations the fungus has been observed in the phloem, xylem, cortex and root hairs. It

has been isolated from the primary, secondary and tertiary roots, and from stems, leaves, pedicels, melon flesh and seeds. In addition it has been observed in the water ducts of healthy appearing runners and has been isolated from the roots and runners of healthy appearing plants.

In making a study of the symptoms of watermelon wilt on the roots, it was found necessary to remove the root system from the soil in order to determine the point or points of infection. Plants exhibiting all stages of wilt were examined. Some had been dead a day or more, some had only one wilted runner, some were showing the very first signs of wilt, while others appeared normal and healthy. Many different varieties were examined, including Kleckley Sweet, Tom Watson, Halbert Honey, Irish Gray, Excel, Thurmond Gray, Stone Mountain and Conqueror. In addition, isolations were made from vines of the preserving citron. The root systems of more than 200 plants were examined during the summer of 1926. The method of removal was somewhat as follows, varying in some cases to meet certain needs.

A circular trench, about 8 inches wide and 2 feet deep, was dug around the plant to be studied. The ball of earth holding the root system was then loosened and the soil shaken off. It was never possible to secure the entire root system in this manner. Some of the smaller side roots always broke off, but since the soil was very loose and fine it was not difficult to get most of the roots. Record was kept of the above ground symptoms in each case and in the event that only a few runners were wilted, record was made to show the relation of these runners to necrotic areas on the root system.

These root systems were taken into the laboratory and rough sketches made indicating, when possible, the lesions found following the invasion of the fungus. After being disinfected for 5 minutes in a 1-1000 solution of mercuric chloride, followed by a series of washings in sterile water, isolations were made from all parts of the root system and many of the above ground parts of the plants. Platings were made on acidified potato dextrose agar, and readings made after two or three days.

Table II shows that in every case where above ground wilting occurred the primary root yielded the organism. This is indicative of infection of below ground parts in the majority of cases. It is also evident that if only one side of a plant is dead, the healthy appearing portion is harboring the organism about 84 percent of the time. Further, it is shown that plants which appear perfectly normal and healthy, showing none of the symptoms of wilt, often have necrotic areas on their roots and are also harboring the organism in the above ground tissues. Tims (24) states that healthy appearing cabbage seedlings growing in soil infested with *Fusarium conglutinans* may harbor the organism until such time as conditions make it possible for the

fungus to overcome the host. Edgerton (4) states that healthy appearing tomato plants growing in soil infested with *F. lycopersici* may be harboring this organism. Cromwell (3) found *F. tracheiphilum* in healthy appearing soybean plants. Mycelium and microconidia of what appeared to be *F. niveum* have been found microscopically in the vascular tissues of healthy appearing watermelon plants. Isolation, inoculation and re-isolation have shown that the Conqueror strain, altho quite resistant to wilt under Iowa conditions, may harbor *F. niveum* in healthy appearing plants.

It is also evident from table II that all root lesions yielded *Fusarium niveum* and that where any root necrosis occurred the primary root, altho showing no outward evidence of disease, was harboring the organism. The organism has been recovered from roots growing at a depth of 3 feet in the soil. In such cases, however, root lesions were usually evident nearer the soil surface. It does not necessarily follow that the fungus gained entrance to the root 3 feet below the soil surface, but the evidence suggests that once the fungus gains entrance it may rapidly permeate the vascular system of the root.

Taubenhaus (23) reports the isolation of *Fusarium niveum* from the soil 48 inches below the surface, but, as will be shown

TABLE II. PRESENCE OF *FUSARIUM NIVEUM* IN WATERMELON PLANTS SHOWING DIFFERENT EXTERNAL SYMPTOMS

Plant	Symptoms	Result of isolations							
		Roots						Stems	
		Primary		Secondary		Tertiary			
		Healthy	Necrotic	Healthy	Necrotic	Healthy	Necrotic	Healthy	Wilted
1	3 wilted runners—others healthy	*		0	*	0		*	
2	Entire plant wilted	*	*	*	*	*			*
3	Half of runners wilted	*		*	*	*		0	*
4	First symptoms of wilt	*		0	*	*			*
5	First symptoms of wilt	*	*	*	*	*	*		*
6	Half of runners wilted	*		*	*	*		*	*
7	Only one runner alive	*	*	0	*	*	*	*	*
8	Half of runners dead	*		*	*			0	*
9	One runner wilted	*		*	*	0		*	*
10	One runner starting to wilt	*	*	0	*	0		*	*
11	Half of runners wilted	*	*	*	*	*	*	*	*
12	Half of runners wilted	*		0	*	0		*	*
13	Healthy appearing plant	*		0	*	*	*	*	
14	Plant dead and runners brittle	*	*	*	*	*	*		*
15	Stunted, healthy appearing plant	*		0	*	0		*	
16	3 runners wilted—others healthy	*		*	*	0		*	*
17	Half of runners wilting	*		0	*	0		*	*
18	Runners wilted and brittle	*	*	*	*	*	*		*
19	All runners wilting	*		*	*	0			*
20	All runners wilting	*	*	*	*	0			*
21	Only one runner wilted	*		0		0	*		
22	Only one runner wilted	*			*	0			*
24	One runner partially wilted							*	*

*Indicates that the fungus was isolated.
[0]Indicates failure to isolate the fungus.

TABLE III. PRESENCE OF *FUSARIUM NIVEUM* IN ALL PARTS OF WATERMELON PLANTS

Source	No. of platings	Percent Fusarium
Wilting Plants		
Crown	243	84
Tips of runners	447	96
Primary roots	576	69
Secondary roots	1,012	81
Tertiary roots	664	42
Melon flesh	205	51
Melon pedicels	481	91
Stem near crown	335	86
Melon seeds	1,318	8
Healthy Plants		
Stem near crown	136	34
Tips of runners	235	24

later, this organism is a strict aerobe. Hence, it is doubtful if the organism could live at the extreme depth from which Taubenhaus reports its isolation. The author has never found lesions at such a depth in the soil, altho the organism has been isolated from roots 3 feet below the soil surface.

Another interesting case is the following: Quite commonly a runner is found which is healthy from the crown of the plant outward for a distance of 2 or 3 feet, but which shows symptoms of wilt at its tip. In such cases it has been found that the runner has taken root and that these new roots have become infected. This infection is responsible for the wilting of the runner outward, while the portion between the new roots and the crown remains healthy, since it is nourished by the main root system of the plant.

The results obtained from isolations from 231 plants from July 10 to September 1, 1926, together with the source of the isolations are shown in table III.

EVIDENCE THAT WATERMELON WILT IS SEED BORNE

Record is made here of successful attempts to isolate *Fusarium niveum* from the melon flesh and seeds. That watermelon wilt is seed borne is the belief of many growers at Conesville and Muscatine who claim that the fungus came in on seed.

Edgerton (4) found that spores of *Fusarium lycopersici* would remain viable on the surface of tomato seeds for about three months. It remained for Elliott (5), however, to show that tomato seed naturally infested with *F. lycopersici* would carry the disease from field to field. Elliott (6) also showed that the cotton wilt fungus, *F. vasinfectum*, was seed borne. Stokdyk (22) reported unsuccessful attempts to isolate *F. conglutinans* from cabbage seed, and Fulton (7) found that watermelon wilt was spread by wilt infested seed.

Since the watermelon wilt organism is found on melon pedicels and in melon flesh so commonly, and since seed is often ex-

tracted by machinery from melons grown in wilt infested fields, it is not unreasonable to suppose that the organism might be seed borne. Gardner (8) has shown in the case of cucumber anthracnose that the seed coat is covered with cellulose rods or hairs, which, upon drying, become closely appressed to the seed and protect the spores of the organism. Some of these hairs are also present on watermelon seeds. In view of the work of Maneval (9), who found that *Fusarium niveum* would remain viable for eight years in a test tube, it may be found that watermelon wilt will survive on the seed for some time.

During the past eight months considerable evidence has been accumulated to show that *Fusarium niveum* is often present on watermelon seeds. The first evidence of this was obtained by isolating the organism from the seeds of melons attached to wilting vines. The organism was recovered from about 8 percent of the seeds plated.

Following this point further, seeds of several varieties of watermelons were obtained from three seed companies, one each in Texas, Florida and Georgia. In order to determine whether or not these seeds were carrying *Fusarium niveum* two sets of experiments were outlined. In one set attempts were made to isolate the organism directly from the seeds by the poured plate method. In the second set, the seeds were planted in steam sterilized soil.

On January 14, 22 5-inch pots were filled with steam sterilized soil and seeds of 10 varieties of watermelons secured from three seed companies and one grower were planted. (See Table IV.

The data in table IV add more evidence to the belief that watermelon wilt is seed borne. These data show that the organism was probably present on at least 9 of the 264 seeds planted.

TABLE IV. SHOWING THE PREVALENCE OF *FUSARIUM NIVEUM* ON WATERMELON SEEDS FROM VARIOUS SOURCES

Variety	Source	Total plants	Plants wilted	Percent wilt
Augusta Rattlesnake	Georgia	11	0	0
Irish Gray	Georgia	12	1	8
Irish Gray	Florida	14	0	0
Irish Gray	Texas	9	0	0
Watson	Georgia	11	0	0
Watson	Florida	15	0	0
Watson	Texas	14	0	0
Stone Mountain	Georgia	9	1	11
Halbert Honey	Georgia	16	3	14
Halbert Honey	Texas	8	1	12
Kleckley Sweet	Georgia	11	0	0
Kleckley Sweet	Florida	10	0	0
Kleckley Sweet	Texas	18	1	5
Florida Favorite	Georgia	11	0	0
Florida Favorite	Florida	9	0	0
Florida Favorite	Texas	16	0	0
Thurmond Grey	Georgia	18	2	11
Georgia Rattlesnake	Florida	15	0	0
Kleckley Sweet	Iowa Grower	33	0	0
Total		264	9	3.4

These seeds came from one seed company in each of three states and from one grower in Iowa.

To determine further the relation of *Fusarium niveum* to watermelon seeds from various seed companies, isolations were made by planting the seeds on poured plates of potato dextrose agar. In most cases such organisms as Aspergillus, Penicillium, Rhizopus, Mucor and Alternaria almost completely covered the plates in three or four days, but in a few cases a Fusarium was isolated which proved pathogenic to watermelon seedlings.

In 1926 wilt appeared in two hills of watermelons in a field that was reported to have been in sod for at least 15 years. It was broken up and planted to corn in 1925 and to watermelons in 1926. The grower stated that this field had never been planted to watermelons. These two hills were widely separated, and a Fusarium species recovered from them proved pathogenic to watermelon seedlings. The seed which had been used to plant this field in 1926 had not been treated. This is more evidence that the disease is seed borne, altho possibly the organism may have been carried in by the wind.

LONGEVITY OF FUSARIUM NIVEUM IN THE SOIL

Mention should be made of the longevity of *Fusarium niveum* in the soil. In late July, 1926, the writer was called to inspect a 60-acre field of watermelons on Muscatine Island. According to the grower this field had produced its last crop of watermelons in 1910, a slight amount of wilt having been observed that year. The grower had thought it best not to plant this field to watermelons for the next few years, and finally the field had become overgrown with weeds, no cultivated crop of any kind having been grown during the 16 years from 1910 to 1926. A group of growers planted this field to watermelons in 1926, thinking they would not be troubled with wilt. At the time of the writer's first visit to this field, late in July, wilt was just beginning to appear. On a second visit, about August 15, it was found that about 50 percent of the plants were partially or wholly infected. It would appear that *F. niveum* can live for at least 16 years in the soil in the absence of watermelon plants.

PHYSICAL FACTORS IN RELATION TO INFECTION

RELATION OF TEMPERATURE TO MYCELIAL GROWTH

Preliminary tests with *Fusarium niveum* growing on potato dextrose agar indicated that it grew best at fairly high temperatures. To determine further this point, two liters of Richard's solution containing 3 percent agar were made up and divided equally among 10 500 cc. Erlenmeyer flasks, after which the medium was sterilized for 20 minutes at 15 pounds pressure.

Eighty plates were poured on February 9, 1926, and after inoculation with uniform quantities of mycelium of *F. niveum*, 10 plates were distributed to incubators ranging in temperature from 8°C. to 40°C. Readings were taken at the end of three, five and seven days by measuring the diameter of each colony in centimeters, with the results which appear in table V.

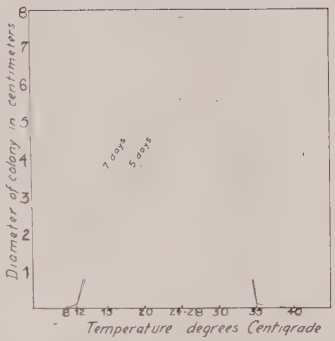

Fig. 5. Graphic representation of the relation of temperature to rate of mycelial growth of *Fusarium niveum*.

Under the conditions of the experiment the optimum temperature for the growth of *Fusarium niveum* lies between 24°C. and 32°C. Growth is very slow at 12°C., the minimum lying between 8°C. and 12°C. Growth is also slow at 35°C. The maximum was not determined, but it is not much above 35°C. See Figs. 5 and 6.

RELATION OF OXYGEN TO MYCELIAL GROWTH

Not only is the influence of temperature on the development of *Fusarium niveum* important, but also the relation of diminished oxygen supply. To determine this relation 25 potato dextrose agar slants were inoculated with *F. niveum*. The oxygen was removed from 20 of these by the action of pyrogallic acid and sodium hydroxide, while the remaining five were allowed to grow normally. At the end of five days no growth occurred in the oxygen-free air, while profuse development was evident in the controls. At this time oxygen was permitted to enter 5 of the 20 tubes and at the end of five more days good growth was evident. The remaining 15 in oxygen-free air had made no growth. At this time, 10 days after inoculation, oxygen was admitted to five tubes and in five days good growth was again evident. In like manner at the end of 15 and 20 days respectively, oxygen was admitted to five tubes and in every case good

TABLE V. RELATION OF TEMPERATURE TO RATE OF MYCELIAL GROWTH OF *FUSARIUM NIVEUM*

Degrees C.	Mycelial growth in centimeters		
	Three days	Five days	Seven days
8	0	0	0
12	0	Trace	Trace
15	Trace	1.6	2.3
20	1.6	3.8	5.0
24-28	2.1	5.5	7.7
30	4.0	5.2	7.8
35	Trace	Trace	Trace
40	0	0	0

Fig. 6. The relation of temperature to the rate of mycelial growth of *Fusarium niveum*. A—8°C, B—12°C, C—15°C, D—20°C, E—24-28°C, F—30°C, G—35°C, H—40°C.

growth was evident within five days after the admission of oxygen. How long the fungus will remain alive in oxygen-free air has not been determined, but it may be classified as an aerobe.

RELATION OF HYDROGEN-ION CONCENTRATION TO MYCELIAL GROWTH

Since watermelon wilt occurs in different soil types it seemed advisable to determine the acidity and alkalinity range for the growth of the fungus. Accordingly, on January 19, 1927, two liters of Richard's solution containing 5 percent agar, were made up. Two hundred cubic centimeters of this medium were placed in each of 10 500 cc. Erlenmeyer flasks and sterilized for 15 minutes at 20 pounds pressure.

After these media were sterilized, cooled and melted, adjustments of the contents of each flask were made by adding sufficient quantities of sterile dilute H_2SO_4 or of NaOH to bring about the desired acidity or alkalinity. Flasks of media were adjusted so as to obtain the following hydrogen-ion concentrations: pH 3.0, 3.3, 3.8, 4.2, 4.6, 5.4, 5.8, 6.8 and 8.4. Ten plates were poured from each of these nine flasks and inoculated with a uniform quantity of mycelium of *Fusarium niveum*. These 90 plates were incubated at 30° C. The rate of growth was determined at

TABLE VI. THE RELATION OF HYDROGEN-ION CONCENTRATION TO THE RATE OF MYCELIAL GROWTH OF *FUSARIUM NIVEUM*

pH	Average diameter of colonies in centimeters				
	Two days	Three days	Four days	Five days	Six days
3.0	0	0	0	Trace	Trace
3.3	0	0	Trace	0.4	1.10
3.8	0	.80	1.61	2.64	3.84
4.2	0.85	1.85	2.95	4.43	5.66
4.6	1.66	3.00	4.44	5.93	7.23
5.4	2.33	3.85	5.23	6.93	8.00
5.8	1 55	3.24	4.57	6.39	7.63
6.8	1.30	2.85	4.13	5.57	7.10
8.4	1.60	3.10	4.00	5.00	5.80

the end of two, three, four, five and six days by measuring the diameter of each colony. See table VI and Figs. 7 and 8.

The data in table VI show that *Fusarium niveum* is tolerant of a wide range of acids and alkalis. The fungus may grow at pH 3.0, the rate of growth increasing with a decrease in the acidity of the medium. The fungus grows most rapidly near pH 5.4, the rate decreasing slightly at pH 5.8, 6.8 and 8.4.

These data suggest that the hydrogen ion concentration of the soil probably has little effect upon the growth of *Fusarium niveum*. Practically all the soil used for watermelons in southeast Iowa is slightly acid. Whether the application of lime to the soil in large quantities would tend to retard the growth of the organism or tend to render plants more tolerant of the fungus remains to be determined. Edgerton (9), working with tomato wilt caused by *F. lycopersici*, found that heavy applications of lime would retard the appearance of wilt.

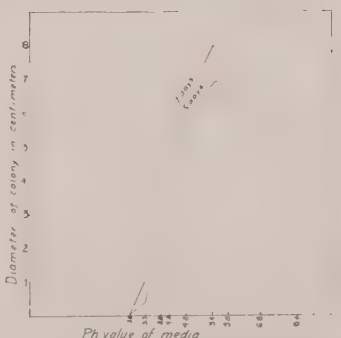

Fig. 7. Graphic representation of the relation of the hydrogen ion concentration to rate of mycelial growth of *Fusarium niveum*.

STUDIES OF SEEDLING RESISTANCE

Orton (15) states that the Conqueror variety has proven resistant in many localities of the United States—Oregon, however being an exception. The Conqueror is quite resistant under Iowa conditions but is undesirable both from the standpoint of type and quality. The preserving citron is also wilt resistant in Iowa.

Having learned to recognize the symptoms of watermelon wilt

TABLE VII. RELATIVE RESISTANCE TO SEEDLING WILT OF SOME CITRONS AND EDIBLE WATERMELONS

Parent	Percent of plants wilting				
	After 13 days	After 19 days	After 22 days	After 29 days	After 40 days
Preserving citron	0	26	35	60	90
African strain "A"	0	0	0	0	5
African strain "B"	0	0	0	0	5
African strain "C"	0	11	33	44	55
African strain "D"	0	12	19	29	53
African strain "E"	0	7	7	22	32
Stone Mountain	12	17	50	80	95
Control (Sterile soil)	0	0	0	0	0

in the seedling stage, it was thought advisable to test the relative resistance of the hybrids resulting from crosses made in 1925 and 1926 and to test the relative seedling resistance of the various citrons, the Conqueror strain and some commercial varieties of watermelons. Seed of the livestock citron, preserving citron and several of the South African citrons was available, as well as seed of 33 hybrids resulting from crosses between citrons and edible watermelons.

Accordingly, on January 7 a series of 54 5-inch greenhouse pots were filled with sterile soil and infested with a small quantity of a giant culture of *Fusarium niveum* growing on sterile oats. Seed of the preserving citron and South African citron was disinfected for 15 minutes in a 1-1000 solution of mercuric chloride, washed twice in sterile distilled water, soaked 20 hours in sterile water and planted at the rate of 10 seeds per pot. Six pots were planted with seed of seven different citrons in addition to six pots of Conqueror and six of Stone Mountain, a commercial

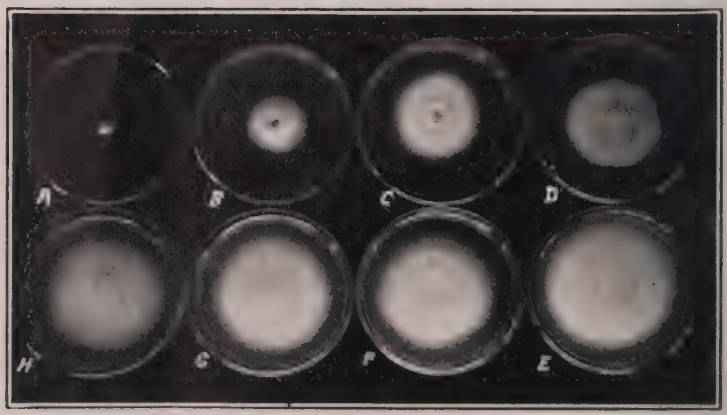

Fig. 8. The relation of hydrogen ion concentration to rate of mycelial growth of *Fusarium niveum*. A—pH 3.3; B—3.8; C—4.2; D—4.6; E—5.4; F—5.8; G—6.8; H—8.4.

susceptible variety. Data were taken on the time of emergence and the rate of wilting from 6 to 40 days. (See table VII.)

The data in table VII suggest that the relative resistance of varieties may be measured in the seedling stage, provided readings on the percent of plants wilting are taken before the plants are 30 days old. At the end of 13 days Stone Mountain was the only variety which was wilting. At the end of 22 days 50 percent of the Stone Mountain plants were wilted, while the resistant varieties averaged only 16 percent wilt. After 29 days 80 percent of the Stone Mountain plants were wilted while the resistant varieties averaged only 22 percent. After 40 days, however, as many plants of the preserving citron had died as of the Stone Mountain. This suggests that varieties highly resistant under field conditions will finally, upon being subjected to conditions favoring the development of the pathogen, succumb in the seedling stage to the same degree as susceptible sorts.

Fig. 9. Seedling resistance of African 1 B-1, one of two strains resistant to seedling wilt. These plants are growing in heavily infested soil. They lived, bloomed and formed small melons under these conditions.

Mention should be made, however, of African strains "A" and "B." African strain "A" showed no infection during the first 29 days, while African "B" showed none for 22 days. After 40 days these strains showed only 5 percent infection, while the preserving citron and Stone Mountain showed 90 and 95, respectively. Many of the plants of African strains "A" and "B" bloomed and formed fruit while still growing in the pots. Fig. 9 shows African "A" after 58 days exposure to infection.

LITERATURE CITED

(1) Barker, H. D.
1923. A Study of Wilt Resistance in Flax. Tech. Bul. Minn. Agr. Exp. Sta. 20.

(2) Crawford, R. F.
1923. Fungi Isolated from the Interior of Cotton Seed. Phytopath. 13:501-503.

(3) Cromwell, R. O.
1919. Fusarium Blight of the Soy Bean and the Relation of Various Factors to Infection. Res. Bul. Nebr. Agr. Exp. Sta. 14.

(4) Edgerton, C. W. and C. C. Morland.
1920. Tomato Wilt. Bul. La. Agr. Exp. Sta. 175.

(5) Elliot, J. A. and R. F. Crawford.
1922. The Spread of Tomato Wilt by Infected Seed. Phytopath. 12:428-434.
(6) ———
1922. Cotton Wilt a Seed Borne Disease. Phytopath. 12:50-51.
(7) Fulton, H. R. and J. R. Winston.
1913. Watermelon Wilt Spread by Contaminated Seed. Bien. Rept. N. Car. Agr. Exp. Sta. 1913-1914: 48-51.
(8) Gardner, M. W.
1918. Anthracnose of Cucurbits. Bul. U. S. Dept. of Agr. 727.
(9) Maneval, W. E.
1924. Longevity of Cultures of Fusaria. Phytopath. 14:408-410.
(10) Melhus, I. E. and O. H. Elmer.
1925. Diseases of Cucumbers and Melons in Iowa. Circ. Ia. Agr. Exp. Sta. 99.
(11) Orton, W. A.
1902. On the Breeding of Disease Resistant Varieties. Proc. Inter. Conf. on Plant Breeding and Hybridization. Mem. 1:41-54.
(12) ———
1904. Plant Breeding as a Factor in Controlling Plant Diseases. Amer. Breeders Assoc. 1:69-72.
(13) ———
1907. A Study of Disease Resistance in Watermelons. Science N. S. 25, 288.
(14) ———
1908. On the Theory and Practice of Breeding Disease Resistant Plants. Amer. Breeders Assoc. 4:145-156.
(15) ———
1911. The Development of Disease Resistant Varieties of Plants IV. Conf. Internationale de Genetique, Paris. 247, 265.
(16) Porter, D. R.
1927. Watermelon Wilt Infection Studies. Phytopath. 17:9-10. (Abst.)
(17) Rosen, H. R.
1925. Fusarium Vasinfectum and the Damping off of Cotton Seedlings. Phytopath. 15:8:486-488.
(18) Smith, E. F.
1894. The Watermelon Disease of the South. Proc. Amer. Assoc. Adv. Sci. 43:289.
(19) ———
1895. The Watermelon Wilt and Other Wilt Diseases due to Fusarium. Proc. Amer. Assoc. Adv. Sci. 44:190.
(20) ———
1897. The Spread of Plant Diseases. A Consideration of Some of the Ways in Which Parasitic Organisms are Disseminated. Trans. Mass. Hort. Soc. 1897.
(21) ———
1899. Wilt Disease of Cotton, Cowpea, and Watermelon. Bul. U. S. D. A. Div. Veg. Path. and Phys. 17.
(22) Stokdyk, E. A.
1924. Progress Report on Cabbage Yellows Investigation in Kansas. Phytopath. 14:24. (Abst.)
(23) Taubenhaus, J. J.
1920. Wilts of the Watermelon and Related Crops. Tex. Agr. Exp. Sta. Bul. 260.
(24) Tims, E. C.
1926. The Influence of Soil Temperature and Soil Moisture on the Development of Yellows in Cabbage Seedlings. Jour. Agr. Res. 33:10.